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Storage and ultraviolet-induced tissue stress effects on fresh-cut pineapple†

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Abstract: The effect of UV-induced stress on the volatile aroma compounds in cut pineapple was compared with that of storage at 4 °C for 24 h. Eighteen volatile compounds were identified by solid-phase microextraction (SPME) in fresh-cut pineapple. Methyl-2-methylbutanoate, methyl hexanoate, methyl 5-hexenoate, ethyl hexanoate and ethyl 5-hexenoate were the major aroma compounds. Storage at 4 °C for 24 h, and exposure of cut fruit to UV radiation for 15 min caused a considerable decrease in the concentration of esters and increase in the relative amount of copaene. This sesquiterpene, when added to crushed cantaloupe melon (0.1 mg g^{-1}), inhibited microbial growth in the fruit over a period of 24 h at 20 °C. *Cis*- and *trans*-ocimene were present in the fruit but their production was not photo-induced by UV irradiation. Ocimene, however, was a potent antimicrobial agent that killed microorganisms when added to the crushed fruit and stored at 20 °C for 24 h. The results indicate that sesquiterpene phytoalexins could contribute to the defense mechanism in wounded pineapple tissue.
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Keywords: *Ananas comosus*; phytoalexins; pineapple; minimally processed; terpenes; fruit; volatiles; SPME

INTRODUCTION

Pineapple (*Ananas comosus*) is the world's most popular non-citrus tropical and subtropical fruit. The main commercial outlet is as fresh uncut fruit. However, minimal processing serves as a means of reducing the amounts of surplus fruit and makes fruit consumption convenient for consumers.[1] Consumption of pre-cut fruits, including pineapples, has increased considerably because of the convenience offered to consumers by these fresh-cut products.[2] The shelf life of cut fruit is considerably less than that of the intact uncut fruit. Consequently, there has been considerable interest in changes in the fruit and/or processing conditions that influence the shelf life of cut fruits.[3–5]

We recently demonstrated that storage of cut cantaloupe melon at 4 °C caused a considerable decrease in the concentration of esters and synthesis of the phytoalexin terpenoid compounds, β-ionone and geranylacetone, over a period of 24 h.[6,7] The changes in volatile aroma compounds were similar to those that occurred as a result of the exposure of the cut tissue to UV light.[8] The loss of ester compounds during storage of cantaloupe melon, which is apparently responsible for the development of staleness in the stored refrigerated fruit, thus appears to be the result of a stress-induced defense response in the cut fruit as an adaptation process to tissue exposure and cell disruption.

Analysis of pineapple flavor components and their precursors has been widely discussed in the literature. Most of the methods reported involved distillation, solvent extraction and/or headspace gas chromatography.[9,10] Several classes of compounds including hydrocarbons, esters, sulfur-containing compounds, lactones, carbonyl compounds, alcohols and phenols have been identified.[10,11] Of these compounds, esters are the most abundant and probably the most important pineapple aroma compounds.[12] The objective of this study was to identify the volatile aroma compounds in pineapple using a low temperature solid phase microextraction (SPME) GC-MS method, determine the effect of storage of cut pineapple on the volatile compounds, and compare these to the effect of UV-induced biological tissue stress in cut pineapple.

EXPERIMENTAL

Sample preparation

The pineapple (*Ananas comosus* (L) Merr) cultivar used was Delmonte MD2 purchased from a local supermarket. The fruit was first sliced longitudinally into two halves and one half was further cut equatorially. Slices (*ca* 1–2 mm thick) were obtained from the exposed cut end. The changes in the slices were measured because the disrupted cells

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and intercellular spaces between the first few layers of cells are most affected by fresh-cut processing.[13] Changes in volatile aroma compounds in cut fruit slices (about 1 mm thick) were determined. The fruit slices were transferred into glass Petri dishes after cutting off approximately 2 mm around their edges, including the skin. Analysis of the fresh-cut fruit for volatile aroma contents was performed immediately. Fruits prepared for storage were sliced, placed in glass petri dishes and stored at 4 °C. Replicate samples from three separate pineapples were removed and prepared for analysis. Samples to be treated with UV radiation were transferred into petri dishes and placed under ultraviolet light (Fotodyne (Hartland, WI) 3–3000) for 15 min. Petri dishes containing the samples were placed on a glass beaker so that the samples were approximately 5 cm from the light source. Fruit preparation for GC-MS analysis involved finely chopping treated fruit and the control fresh-cut fruit (3 g), and transferring the resulting slurry into a vial (20 ml) containing NaCl (1 g) and into which a magnetic stirring bar was inserted. The vial was fitted with an aluminum septum cap and sealed. Benzothiophene (1.55 μg kg^{-1}) in methanolic solution was added as an internal standard by injection onto the pulverized fruit and mixed thoroughly by agitation.

GC-MS analysis

Volatile components of the fruit were extracted by headspace solid-phase microextraction (SPME) using a fused silica fiber (Supelco, Bellefonte, PA, USA) coated with a 100-μm layer of dimethylpolysiloxane conditioned by inserting it into the GC inlet for 2 h prior to use for volatile compound adsorption. With the vial containing the fruit mixture inserted in a water bath (30 °C), the sample was stirred for an initial period of 30 min, after which the SPME fiber was inserted into the sample headspace for 15 min while stirring continued. The fiber was desorbed in the GC inlet at 250 °C for 4 min.

GC-MS analysis was performed on a Hewlett-Packard (Albertville, MN) HP-6890 series system utilizing a HP-5 MS Crosslinked 5% phenyl methyl siloxane (30 m × 0.25 mm × 0.25 μm) column. The GC was operated in a splitless mode with helium as the carrier gas (25 psi). The carrier gas flow rate was 20.8 ml min^{-1}. The oven was programmed with an initial temperature of 60 °C ramped to 215 °C at a rate of 8 °C min^{-1}, then to 260 °C and held for 15 min. The mass spectrometer was operated in scan mode from 40 to 400 amu, using 70 eV electrons for ionization. Compounds were identified from their retention times using a commercially available library (HP ChemStation software), reference compounds, and MS fragmentation patterns. The concentrations of individual compounds were determined by comparing their peak areas with the peak area of the internal standard. Total volatile compounds emitted from the fruit were calculated as the total area of the chromatogram during the first 15 min relative to the area of the internal standard. The combined total area of the identified esters was also used to estimate total ester contents of the fruit samples.

Determination of microbial activities

Nutrient agar (Difco, Detroit, MI, USA) was prepared and used for aerobic microbial enumerations. Using a sterile technique, fruit slices (3 g) were finely chopped and separately mixed with 300 μl ocieme and copaene (Sigma-Aldrich, St Louis, MO, USA) respectively. Replicated samples of the mixture were incubated at 20 °C for 24 h after which they were suspended in sterile water (190 ml) by swirling. After a series of dilutions, 0.1 ml of the fruit suspension was overlaid on the surface of a nutrient agar plate. Duplication was made for each dilution. Bacterial colonies were counted after 24 h of incubation at 30 °C. Colony forming units (CFUs) per gram of melon were calculated. Controls were pineapple slices that were chopped and incubated for 24 h at 20 °C without addition of the terpenoid compounds and a set that was prepared for plating immediately after cutting, without either UV treatment or incubation for 24 h at 20 °C on addition of the terpenoid compounds.

RESULTS AND DISCUSSION

Eighteen volatile aroma compounds were identified in fresh-cut pineapple (Table 1). Most of these compounds were esters, and were previously reported to be present in pineapple. Extraction of volatile compounds was carried out in this study at a relatively low temperature (30 °C) to minimize method-dependent changes such as those induced by heat stress on the fruit tissue. The delicate balance in volatile composition is better preserved but the low temperature precluded the extraction of heavier and less volatile compounds. The major compounds identified in the fruit were methyl 2-methylbutanoate, methyl hexanoate, methyl 5-hexenoate, ethyl hexanoate and ethyl 5-hexenoate. Some of the compounds, methyl and ethyl 3-methylthiopropionate, ethyl 2-methylbutanoate, and ethyl hexanoate, are believed to be important contributors to pineapple aroma.[11,12,14] The presence of *trans*-β-ocimene in pineapple reported by Takeoka *et al*[10] appears to be the only reported identification of this terpenoid compound in pineapple. This compound, which was emitted into the headspace of the whole intact fruit and vacuum steam-distilled blended pulp, was absent from the headspace analysis of the blended pulp. The *cis* and *trans* isomers of β-ocimene were identified by the SPME extraction method used in this study. In addition, a closely related octatriene compound (RT = 5.08; M^+ = 136; 80 (56), 41(24), 44 (21), 106 (19), 121 (18), 53,67 (13), 55 (11), 136 (8), 74,103,120 (5)) was tentatively identified. The presence of the isomers could also be cultivar specific. Ocimene, an acyclic monoterpene, is

Table 1. Volatile compounds, expressed as amounts relative to the internal standard, in fresh-cut pineapple, cut pineapple stored at 4 °C for 24 h and cut pineapple exposed to UV light for 15 min

Retention time	Compound	Fresh-cut	24 h	UV
0.94	Ethyl acetate	0.1	0.04	0.04
0.98	Ethyl propionate	0.03	0.02	0.01
1.27	Methyl butanoate	0.19	0.14	0.12
1.56	Methyl 2-methylbutanoate	1.02	0.36	0.21
2.11	Ethyl 2- methylbutanoate	0.32	0.14	0.1
2.91	Methyl hexanoate	2.27	1.14	0.72
3	Methyl 5-hexenoate	3.25	0.72	0.8
3.94	Ethyl hexanoate	1.24	0.51	0.26
4.01	Ethyl 5-hexenoate	2.11	1.04	0.5
4.41	Methyl 3(methylthio)propanoate	0.28	0.27	0.15
4.72	*cis*-Ocimene	0.11	0.1	0.12
4.99	*trans*-Ocimene	0.62	0.32	0.27
5.08	Octatriene compound	0.53	0.5	0.39
6.21	Ethyl 3-(methylthio)propanoate	0.36	0.33	0.18
6.55	Methyl (*E*)-4 octenoate	0.23	0.24	0.14
6.73	Methyl octanoate	0.79	1.01	1.03
8.23	Benzothiophene (standard)	1	1	1
9.31	Methyl 5-acetoxyhexanoate	0.91	1.02	0.77
10.48	Sesquiterpene compound	0.22	0.37	0.55
11.62	Copaene	0.71	1.3	1.5

an essential oil that is widely distributed in plants and contributes to the aroma of many fruits.[15,16]

Refrigerated storage of the fruit for a period of 24 h caused a decrease in esters relative to the freshly cut fruit. The highest losses occurred in the major compounds, ranging from about 60% of ethyl hexanoate to over 75% of methyl 5-hexenoate. Production of copaene also occurred, increasing by 83%, as well as that of a tentatively identified sesquiterpene (RT = 10.48; $M^+ = 204$; 148 (100), 91 (78), 133 (77), 105 (74), 161 (60), 119 (51), 190 (40), 79,93 (26), 41 (23)) which increased by 70% over the amount present at the time the fruit was cut. Exposure to UV light had a similar degradative effect on the esters and increased production of the sesquiterpene compounds. Storage and exposure to UV radiation, however, caused a decrease in the total monoterpene content (Table 2).

The effects of storage and UV light on the volatile components of cut pineapple were similar to those that occurred in cut cantaloupe melon.[6,8] In both cases, and perhaps in most fruits, the adaptation to wound-induced stress caused changes in volatile aroma components, particularly esters. Esters are known to be involved in stress adaptation to tissue wounding,[17,18] and those found in essential oils exhibit antifungal and antibacterial activities.[19] It was demonstrated in cantaloupe that the loss of esters during storage of the cut fruit is unrelated to microbial effects.[6] Esters, particularly those with acetate as the acyl portion, constitute one of the most important classes of compounds that impact the aroma of fruits. Their loss during storage of cut fruits would alter fruit flavor which is a delicate balance of aroma compounds.

A wide range of terpenoid compounds is found in fruits and vegetables. A number of these have antimicrobial properties and are phytoalexins. The microbial transformation of terpenes could result in reactions such as hydroxylation, epoxidation, hydration of double bonds and reduction.[20] Ocimene appears to undergo hydroxylation.[21] The microbial transformation of sesquiterpenes also results in hydroxylated metabolites.[22] Various sesquiterpene stress metabolites have been isolated from Solanaceae, for example, rishitin and its relatives from potato and tomato, and capsidiol from pepper.[23] Wound stress-induced transformations of sesquiterpene esters to their corresponding furans, aldehydes and lactones may also occur.[24,25] Cyclic and acyclic terpenoid compounds, *cis*- and *trans*-β-ionone, terpinyl acetate and geranylacetone with antimicrobial properties in fruit tissue were produced in response to UV-induced biological stress in cantaloupe melon.[8] The monoterpene compounds, *cis*- and *trans*-ocimene, and the sesquiterpene copaene, identified in this study also have antimicrobial properties. β-Ocimene inhibits the multiplication of fungi, and both Gram-positive and Gram-negative bacteria.[26] Monoterpenes also enhance membrane stability, thus providing rather non-specific protection of photosynthetic and respiratory processes.[27]

Copaene and ocimene, when added to cut pineapple tissue, inhibited microbial growth over a period of 24 h storage at 20 °C (Table 3). Ocimene was a highly potent antimicrobial agent in the fruit. Addition of ocimene killed microorganisms present in

Table 2. Relative amounts of esters, monoterpene hydrocarbons and sesquiterpene hydrocarbons present in fresh-cut pineapple, cut pineapple stored at 4 °C for 24 h and cut pineapple exposed to UV light for 15 min

Compound	Fresh-cut	24 h	UV
Esters	13.1a	6.98b	5.03b
Monoterpene hydrocarbons	1.26a	0.92a	0.78b
Sesquiterpene hydrocarbons	0.93a	1.67b	2.05c
Total Volatiles	15.3a	9.57b	7.86

Table 3. Effect of terpenoid compounds on total microbial counts expressed in colony forming units (CFU) g^{-1} [a]

Sample	Total count (CFU g^{-1})
Fresh-cut	4.2×10^3 (1.0×10^3)[b]
Control	3.8×10^4 (9.5×10^2)[b]
Copaene	2.5×10^3 (7.5×10^2)[b]
Ocimene	0

[a] Fresh cut pineapple was prepared for plating immediately after the fruit was cut while the control was chopped and incubated for 24 h at 20 °C. Terpenoid compounds (0.1 mg g^{-1}) were added to each of the other samples and mixed prior to storage.
[b] Standard errors are in parentheses.

the fresh-cut fruit (4.2×10^3 CFU g^{-1}). Microbial count in the fruit to which copaene was added was 2.5×10^3 CFU g^{-1} over the same period of time. The microbial population in the untreated control was 3.8×10^4 CFU g^{-1}. Wound and UV-radiation-induced formation of sesquiterpenes occur in a number of plant tissues.[28] In solanaceous plants, it was demonstrated that induction of 3-hydroxy-3-methylglutarylCoA reductase (EC 1.1.1.34) is essential for the synthesis of sesquiterpenoid phytoalexins following mechanical injury or pathogen infection. Ocimene can be synthesized through photoinduced charge-transfer isomerization of α-pinene. The ocimene class of monoterpenes can also be made from one or more terpenes of the α-pinene class entirely from reduced carbon pools inside the chloroplasts.[29] This ocimene precursor has not been reported present in the fruit in detectable quantities except in the crown.[10] The lack of effect of UV light on ocimene production might be the result of very low levels of the precursor α-pinene in pineapple. We observed a decrease in the ocimene terpene content of pineapple as a consequence of UV irradiation and storage. *Cis*- and *trans*-β-ocimene also belong to the class of terpenes that are slowly induced by light.[29] Loreto *et al*,[27] based on the lack of response of *cis*- and *trans*-β-ocimene to exposure to high temperatures, low oxygen or fumigation, concluded that they may have a different pathway of formation than other monoterpenes that probably does not involve enzymatic synthesis.

CONCLUSION

The stress adaptation process of fruit to exposure of tissue resulting from fresh-cut processing involves the reduction of volatile aroma compounds, particularly esters, and synthesis of sesquiterpene compounds with phytoalexin properties. In pineapple, production of sesquiterpene compounds occurs, although monoterpenes such as ocimene, that do not increase with storage of the fruit and are not photo-induced, may act as antimicrobial agents in the cut fruit. The loss of esters and changes in volatile aroma composition during storage, including production of terpene phytoalexins, will potentially affect the fruit flavor during storage. The UV-induced production of phytoalexin compounds also demonstrates the potential use of UV radiation to elicit the natural defense mechanism of cut fruits.

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